766-Pos Board B566
Fluorescence Correlation Spectroscopy of Tryptophan-Containing Proteins in Sugar Solutions
Nathan Holman, Yuli Wang, David Sidebottom.
Cryopreservation has become an important avenue of molecular and cellular biology research in recent decades but the mechanism by which even simple cryopreserving agents such as sugar solutions protect biomaterial remains poorly understood. To investigate the behavior of proteins in sugar solutions, we have undertaken a study of sugar solutions using Photon Correlation Spectroscopy (PCS) that reveals a strong tendency for sugars to cluster even at dilute concentrations. To investigate tryptophan-containing proteins dissolved in these solutions, we have also implemented Fluorescence Correlation Spectroscopy (FCS) using a two-photon excitation approach as a selective probe of protein dynamics.

767-Pos Board B567
Reconstruction of Nanostructures in Cells by the Modulation Tracking Optical Method
Enrico Gratton, Michelle A. Digman, Luca Lanzano'.
Resolving biological structures at the nanoscale has been a subject of intense recent research. Essentially two approaches have been proposed, one based on shaping the effective illumination profiles using stimulated emission (STED) and the other using the determination of the center of mass emission by single molecules (PALM and STORM). Both approaches have been shown to produce high quality images with resolution on the order of 20 nm. The STED method is ultimately based on using a raster scan approach to produce an image, which is quite inefficient for imaging objects in 3D and the PALM method is very slow for obtaining 3D structures of the size of microns. We have developed the modulation tracking method which is based on a feedback principle that is capable of producing 3D images with resolution in the 20 nm range. With simulations of 3D objects we show how the images are acquired in 3D and projected in 2D screen and we determine the size limits in imaging very small object. We also show that the modulation tracking method produces images based on the location of the surface of objects with high accuracy and we compare the images obtained by the modulation tracking approach with the 3D reconstruction methods based on the determination of intensity iso-surfaces.

768-Pos Board B568
Advances in Three Dimensional Super Resolution Fluorescence Localization Microscopy
Michael J. Mlodzianoski, Joerg Bewersdorf.
For centuries, the resolution of light microscopes has been restricted by the diffraction limit of light, typically ~250nm. Over the last two decades several methods have been developed to get around these limitations. In particular, FPALM, PALM, and STORM, utilize stochastic optical switching of photoactivatable fluorescent molecules and their sequential localization to generate super-resolution images. After the first generation, which only offered lateral (two dimensional) improvements to resolution, recent developments now achieve 3D super-resolution. The challenge of enhancing resolution in the axial (depth) direction requires breaking the symmetry of the point spread function around the focal plane. Here, we focus on Biplane Fluorescent Photoactivatable Localization Microscopy (BP-FPALM) which utilizes multiplane detection to localize particles with sub-100 nm precision in 3D.
BP-FPALM has been previously described and characterized. Here we demonstrate two color imaging of the mitochondrial matrix and mitochondrial nucleoids with a variety of photoactivatable probes. In addition, we show BP-FPALM's capability of measuring samples of a thickness greater than 5 μm while also containing a large number of localized particles. For image acquisition times longer than a few seconds, sample drift can blur images and reduce resolution. We introduce algorithms to correct and account for any drift.

769-Pos Board B569
Synthesis of Dopamine in Pancreatic β-Cells and Its Impact on Glucose Stimulated Insulin Secretion
Alessandro Ustione, David W. Piston.
Glucose homeostasis is maintained by small clusters of hormone secreting cells in the pancreas: the pancreatic islets. Insulin secreting beta-cells make up ~ 90% of each islet and secrete insulin in a tightly regulated manner. Understanding the mechanisms that regulate insulin secretion is a key factor in developing therapies for type-2 diabetes and metabolic syndrome.
Evidence shows that dopamine inhibits glucose stimulated insulin secretion (GSIS) in vitro, and the effect is mediated by the D2 isoform of the dopamine receptor. Yet, there is no evidence of dopaminergic neurons innervating pancreatic islets, and therefore, the biological relevance of such sensitivity is not clear.
We demonstrate that pancreatic islets can produce dopamine from the circulating precursor L-dopa and that the resulting dopamine is released as an autocrine inhibitory signal to regulate insulin secretion. We generated data from wild type and transgenic mice lacking D2 dopamine receptor or the dopamine transporter (DAT) to understand how dopamine is producing its effects. We monitor islet metabolic activity by imaging of NAD(P)H autofluorescence with two photon excitation and we measure intracellular $[Ca^{2+}]_i$ oscillations by confocal microscopy. We show that dopamine does not affect β-cell metabolic activity. Instead dopamine and L-dopa reduce the frequency of $[Ca^{2+}]_i$ oscillations and this correlates with reduced glucose stimulated insulin secretion (GSIS). This suggests that dopamine receptor activation affects GSIS downstream of glucose metabolism, probably reducing Ca^{2+} influx through the plasma membrane.
This finding provides a new target for drug development to treat type-2 diabetes, and it may explain reported side effects of second generation antipsychotics that result in elevated risk of type-2 diabetes and impaired glucose tolerance.

770-Pos Board B570
Direct Observation of Single Oligomers of the Alzheimer's Amyloid-β Peptide on Live Cell Membranes
Robin Johnson, Joseph Schauerte, Kathleen Wisser, Indu Saluja Igo, Ari Gafni, Duncan Steel.
Oligomeric species of the amyloid-β peptide are strongly implicated in the synaptic dysfunction and neuronal loss seen in Alzheimer's disease. While proposed mechanisms for oligomeric toxicity are abundant and diverse, many involve amyloid-β (Aβ) interaction with cell membranes, either via direct insertion into the cell membrane or by binding to specific cell-surface receptors. One such hypothesis holds that Aβ forms calcium-permeable channels within the membranes of neurons, disrupting homeostasis and triggering an apoptotic signaling cascade. However, both the exact identity of the toxic aggregate or aggregates and the mechanism by which the toxicity is mediated remain undetermined. Study of this system under physiological conditions presents a challenge, as oligomeric forms of the peptide *in vivo* are heterogeneous and metastable, and Aβ itself is normally present in the brain at only nanomolar concentrations. We recently used single molecule spectroscopy to detect stable, conductive low-order Aβ(1-40) oligomers in synthetic membranes exposed to nanomolar levels of the peptide. Similar oligomers may form on the membranes of live cells and, by disrupting cell membrane integrity, contribute to the functional abnormalities and neuronal death observed in Alzheimer's. With single molecule confocal laser scanning microscopy, we have identified small Aβ(1-40) oligomers bound to the membranes of SH-SY5Y neuroblastoma cells after ten minutes' exposure to low (50nM) peptide concentrations. Additionally, we have used single-particle fluorescence intensity measurements to characterize the oligomeric states of these cell-bound aggregates. We find that small oligomers ranging from dimers to octamers form in solution under physiological conditions and that the size distribution shifts towards larger oligomers for Aβ(1-40) on the cell membrane, which may indicate that oligomer growth can occur following membrane binding.

771-Pos Board B571
Macromolecular Crowding and Stem Cell Differentiation
Rafi Rashid, Michael Raghunath, Thorsten Wohland.
Macromolecular crowding (MMC) is a biophysical tool which has been used extensively to enhance chemical reactions and biological processes by means of the excluded volume effect (EVE). The *in vivo* stem cell microenvironment contains macromolecules which are crucial for stem cell self-renewal and cell fate determination. In order to mimic this physiological microenvironment, crowders are included in cell culture medium. We have observed that the *ex vivo* differentiation of human mesenchymal stem cells (hMSCs) into the adipogenic lineage is significantly amplified when a crowder mixture comprising Ficoll 70 and Ficoll 400 is added to the culture medium. Stem cell differentiation is modulated by soluble chemical substances as well as interactions between cells and the extracellular matrix (ECM), and both these external influences may be affected by MMC. Measurements we have performed by fluorescence correlation spectroscopy (FCS) on bulk solutions indicate that crowders change diffusion coefficients via changes in viscosity. Our data do not show that Ficoll additives cause anomalous subdiffusion within a crowder concentration range of 0 to 300 mg/ml. The diffusion of fluorophore-labelled molecules in artificial lipid bilayers and membranes of living cells is not changed by crowders, suggesting that these crowders do not directly alter membrane properties and cell surface signalling. However, we have data to suggest that crowders exert an effect on biomolecular interaction kinetics. We have also observed that crowders are taken up by stem

cells and that they localize to specific compartments. Based upon our observations, we hypothesize that crowders can influence stem cell differentiation by influencing molecular kinetics.

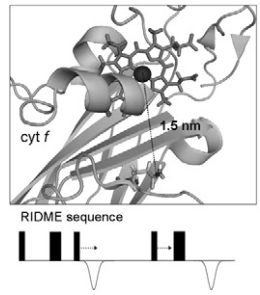
772-Pos Board B572
Volume and Morphological Changes in Red Blood Cells with Pressure Probed by Optical Imaging In-Situ
Silki Arora, Sang Hoon Park, Jennifer Mauser, Debopam Chakrabarti, Alfons Schulte.
Functional properties of living cells depend on the thermodynamic variables temperature and pressure. A unique tool to investigate volume effects on structure and metabolism of the cell is pressure perturbation. We employ a setup that allows visualizing individual live cells at variable pressure in real time. We present measurements of volume changes in red blood cells (RBC) over the pressure range from 0.1 to 200 MPa. Up to a pressure of 35 MPa the size of a healthy erythrocyte remains constant. Over the pressure range from 35 MPa to 200 MPa the lateral diameter decreases linearly and reversibly with a slope of 0.015 micron / MPa, while there are no significant alterations in shape. The RBC deformability is discussed in terms of the cell membrane elasticity and effects of the cytoskeletal network. Our experiments are extended to RBCs infected with the malaria parasite *Plasmodium falciparum*. Here, we observe clear differences in the deformability with pressure and between the compression and decompression curves.

773-Pos Board B573
In Vivo Imaging of Tumor Cell Migration
Christian Weis, Andreas Hess, Tim St. Pierre, Ben Fabry.
The process of metastasis formation involves the migration and 3-D invasion of tumor cells from a primary tumor to distant sites. We propose that the dynamics of the migration and invasion process of magnetically labeled tumor cells can be monitored in animal models over prolonged time periods using magnetic resonance imaging (MRI). Human breast carcinoma cells (MB-MDA-231) were labeled with superparamagnetic Fe_2O_3 iron oxide nanoparticles coated with poly-L-lysine. The particles are readily taken up by cancer cells and stored in intracellular clusters. During cell division, the nanoparticle clusters are divided and split unevenly between daughter cells (mean partitioning fraction 0.85 to 0.15). Nanoparticles are non-toxic, are not degraded by the cell and remain stable for at least 3 weeks. In vitro collagen gel assays show no differences in contractile properties and invasion behavior of magnetically labeled vs. non-labeled tumor cells. MRI of cells suspended in agarose gave a detection limit of the spin-spin-relaxation-rate above the agar background of approximately 70 cells per 1 mm^3. The minimal detection volume of tumor cells in agarose was 25 µl. Detection limit and minimal volume were verified by injecting labeled cancer cells in mice. Spin-spin-relaxation-weighted (T2-weighted) and susceptibility-weighted images show a rapid relaxation behavior and pronounced phase shifts in the vicinity of the injection area compared to control scans. These studies demonstrate the feasibility of the method for long-term observation of cancer cell migration in vivo with MRI.

774-Pos Board B574
Effect of Formalin Fixation on CARS Microscopy of Neural Tissue
Yeon Ho Kim, **Timothy J. O'Leary**, Jeffrey T. Mason.
The cognitive impairments associated with blast-induced mild traumatic brain injury (bmTBI) suggest that exposure to blast may disrupt the connectivity of the fiber tracts that form the neural network of the brain. Coherent anti-Stokes Raman scattering (CARS) microscopy is ideally suited to observe blast-induced structural changes in the myelinated axons of the neural white matter. The sensitivity of the CARS technique to the CH_2 stretching vibrations of the myelin sheath of the axons allows for the label-free imaging of fiber tracts at high spatial resolution with large depth penetration. To date, most CARS studies have been performed on living *in vivo* and *ex vivo* tissues. However, studies of the structural changes associated with bmTBI will require the ability to examine *post mortem* tissue. In this study, we report our findings on the use of CARS microscopy to observe myelin fibers in formalin-fixed mouse and porcine brain tissue. Neural structures including the cerebrum, spinal cord, corpus callosum, and hippocampus were examined. Our findings demonstrate that CARS microscopy can be used to determine fiber orientation and continuity, fiber area percentage, myelin density, and the g-ratio of individual myelin axons in neural tissue fixed in formalin for up to 3 months. In conclusion, we demonstrate that the spectroscopic and morphological artifacts produced by formalin fixation do not interfere with the ability of CARS microscopy to observe and characterize the structure of fiber tracts in formalin-fixed neural tissue.

EPR Spectroscopy

775-Pos Board B575
Distances Between Paramagnetic Metal Centers and Spin Labels in Proteins by Pulsed EPR: The RIDME Method As a New Tool
Sergey Milikisyants, Francesco Scarpelli, Michela Finiguerra, Marcellus Ubbink, **Martina Huber**.



Structure determination in biological systems by electron paramagnetic resonance (EPR) is becoming increasingly popular. Distances in the nm range between spin labels in proteins yield structure restraints[1]. Transition metal-ion centers abound in proteins, but their potential as markers for distance determination is limited by their large g-anisotropies and fast relaxation times.
For many of these centers, the known pulse sequences for e.g. DEER or PELDOR cannot be applied because of excitation bandwidth limitations. The RIDME method[2] circumvents this problem by making use of the spin-lattice relaxation (T_1)-induced spin-flip of the transition-metal ion. Designed to measure distance between such a fast relaxing metal center and a radical, it suffers from a dead time problem. This disadvantage can be avoided by the five-pulse RIDME (5p-RIDME) sequence. An Fe(III)-spin label distance in ths protein cytochrome *f* is determined.[3]
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2. L.V.Kulik, et al. J. Magn. Res. **2002**, *157* , 61–68.
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776-Pos Board B576
The Global Analysis of DEER Data
Eric J. Hustedt.
Previously, global analysis has been successfully used to analyze both continuous wave and saturation transfer EPR data[1-3]. In this work, algorithms have been developed for the global analysis of DEER data. Applications include the analysis of DEER data collected at multiple frequencies or multiple timescales. Analysis of DEER data from the soluble protein CDB3 (MW ≈ 90 kD) has shown that the background DEER signal is not well-fit by an exponential decay due to the large size of the CDB3 dimer. As a result, background correction with an exponential decay prior to analysis results in a poor fit to the data. An algorithm has been developed which explicitly fits the background signal with the radius of the molecule (assuming it is spherical) and the spin concentration as parameters. Using this approach, excellent fits to DEER data can be obtained without prior background correction. Also, DEER data can be globally analyzed to determine changes in the relative populations of components of the distance distribution as a function of experimental conditions. For example, DEER has been previously used to study the structural effects of a proline to arginine mutation at residue 327 of CDB3. Intradimer distances in spin-labelled wild type CDB3 can be fit using a single component distance distribution[4]. The same measurements on P327R CDB3 indicate the mutation induces a second more disordered component in the distance distribution[5]. The global analysis of DEER data collected for multiple spin-labelling sites in both the WT and P327R background is being used to further test this two-component model.
Supported by NIH GM 080513.
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777-Pos Board B577
Nitroxide Spin Label Side Chain Dynamics of Solvent Exposed Sites on Membrane Proteins
Brett M. Kroncke, Justin Kim, Linda M. Columbus.
Understanding the structure and functional dynamics of membrane proteins in their native, hydrophobic environment is key to understanding how proteins function. EPR spectroscopy in combination with site directed spin labeling (SDSL) has the potential to quantify structure and dynamics of proteins of arbitrary weight in their native lipid environment. Several studies have elucidated the structural origins of CW EPR lineshapes of water-soluble proteins; however, CW EPR spectra of nitroxide spin labeled proteins in a detergent/lipid environment have characteristic differences from their water-soluble counterparts. Membrane protein spectra are generally broader and frequently